C_6 bonded silica was especially useful in correlating SFC retention with boiling point for a variety of PAC types.

PAC from fossil fuel and environmental samples have commonly been analysed by SFC, with an application range beyond that of GC and much improved resolution over HPLC methods. Coal-derived PAH containing as many as twelve rings have been eluted in SFC. Identification and quantitation is straightforward with the aid of FID, UV and MS detectors. Very rapid analysis of environmental mixtures of PAC has been demonstrated on a C_{18} column with CO_2/methanol mobile phase and simultaneous pressure, temperature and composition programming.

Many nitrogen-containing PAC cannot be analysed by GC because they are thermally labile, and SFC with TID has been especially useful in allowing identification of hydroxynitrofluorenes and nitropyrenequinones.

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Thin-Layer (Planar) Chromatography

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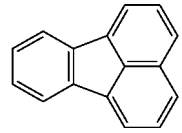
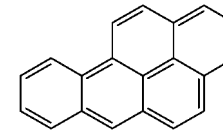
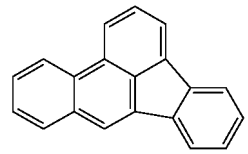
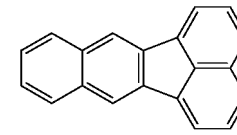
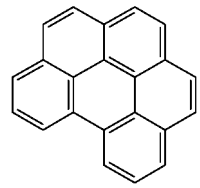
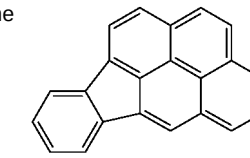
Polycyclic aromatic hydrocarbons (PAH) originate from organic materials (i.e. oil, gasoline, tobacco, garbage, etc.) due to pyrolysis or incomplete combustion. Several hundred different PAH are known, and traces of them can be found nearly everywhere in our environment. In water PAH occur either in solution or adsorbed on to particulate material. As certain PAH are known to be highly carcinogenic, six easily detectable PAH were specified, in order to be used as indicators for the large group of PAH. The structural formulae of these six compounds are given in **Table 1**. In 1971 the World Health Organization (WHO) set the maximum acceptable level of the sum of these six PAH in potable waters at 200 ng L^{-1}. This standard was adopted by the European Community, who fixed the maximum admissible concentration for the sum of the six reference substances in drinking water to 0.2 μg L^{-1}, calculated as carbon.

Thin-layer chromatography (TLC) is one analytical method for the determination of these PAH, in addition to other chromatographic techniques. For standard chromatograms, standard solutions containing fluoranthene with five times the level of the other PAH are usually used. This is based on the fact that fluoranthene is present at approximately five times the level of the other PAH in the majority of water samples. A TLC method using development in two dimensions with two different solvent systems has been known for many years. After separation, the PAH are visualized by irradiation with ultraviolet light, and normally the concentration range of the PAH is visually estimated by comparison with standard chromatograms. In addition to this old semiquantitative TLC method, some modern and more efficient TLC methods have been devised.

The improvement of plate material leading to high performance thin-layer chromatography (HPTLC) allowed the development of efficient procedures for qualitative and quantitative analysis of PAH. A big advantage of the HPTLC methods is the possibility of applying up to 18 samples on to one plate, whereas with the two-dimensional method only one sample could be analysed per plate.

Before the PAH can be separated by TLC, they must be extracted from water samples using, for example, cyclohexane. For a sample volume of 1 L 25 mL of solvent is suitable; larger volumes may be convenient in some cases, e.g. when emulsions form. The solvent extract is concentrated to a small volume and, if necessary, materials interfering in the analysis are removed from the extract using column chromatography with alumina or silica gel. The methods described in the following paragraphs can also be applied to extracts of food, soil, smoke and

Table 1 Names and structures of the six PAH

		Structure		Structure
Name	Fluoranthene		Benzo(a)pyrene	
Abbreviation	F		BaP	
Chemical formula	$C_{16}H_{10}$		$C_{20}H_{12}$	
% Carbon	95.0% C		95.2% C	
Name	Benzo(b)fluoranthene		Benzo(k)fluoranthene	
Abbreviation	BbF		BkF	
Chemical formula	$C_{20}H_{12}$		$C_{20}H_{12}$	
% Carbon	95.2% C		95.2% C	
Name	Benzo(ghi)perylene		Indeno(1,2,3-cd)pyrene	
Abbreviation	BP		IP	
Chemical formula	$C_{22}H_{12}$		$C_{22}H_{12}$	
% Carbon	95.6% C		95.6% C	

other sample material. For some of these samples special, and very time-consuming, clean-up procedures are needed. Therefore TLC is mainly used for the analysis of drinking water.

Thin-Layer Chromatography Method with Development in Two Dimensions

Different adsorbent stationary phases can be used for the separation of PAH. An excellent resolution of the six WHO PAH is obtained on a mixed phase consisting of aluminium oxide G and 40% acetylated cellulose in a 7 : 3 weight ratio. With these plates fluoranthene can be determined in the range of 10–100 ng per spot and the other PAH in the range of 5–80 ng per spot.

Preparation of the TLC Plates

Plates may be prepared as follows using conventional glass TLC plates and a plate-coating apparatus (the quantities given are sufficient for five plates): 28 g aluminium oxide G and 12 g cellulose 40% acetylated are thoroughly mixed with 5 mL ethanol. Clean glass plates 20×20 cm are coated with this mixture at a thickness of 0.25 mm. The coating is air-dried at room temperature for 10–15 min, then activated at 110°C for 30 min. After activation the plates are cooled and stored in a desiccator.

Application of the Sample Extract or Standard Solution

The concentrated extract (0.05–0.1 mL) is spotted in the bottom left corner of the plate, 20 mm from each edge. Microsyringes or capillaries can be used to apply the sample; the diameter of the spot should be about 4–6 mm. The spotting solvent needs about 2–3 min to evaporate at room temperature.

Standard mixtures of the six PAH are applied in the same manner in order to obtain standard chromatograms for the estimation of the PAH concentration.

Development of the Plates

For the chromatographic separation, tanks which have been allowed to equilibrate with the solvent for at least 15 min are used. The migration distance should be about 18 cm in both directions.

- Solvent system 1: a mixture 9 vol *n*-hexane and 1 vol toluene
- Solvent system 2: a mixture of 4 vol methanol, 4 vol diethyl ether and 1 vol water

After separation in solvent system 1, the plate is removed from the tank and dried at ambient temperature in a fume cupboard for 10–15 min. It is then rotated through 90° anticlockwise from the first direction of development and developed in solvent system 2. Afterwards the plate is again removed from the tank and dried in a fume cupboard at ambient temperature for 10–20 min.

The development times are approximately 30 min for solvent system 1 and 90 min for solvent system 2.

Visual Evaluation of the Chromatograms

The plates are irradiated with 366 nm ultraviolet light to visualize the PAH as a series of fluorescent

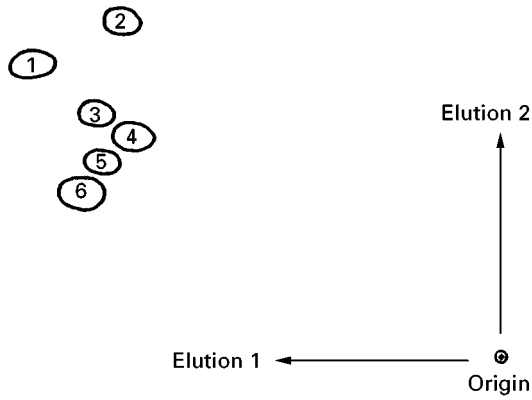


Figure 1 Standard PAH chromatogram viewed under UV light with F (1, turquoise), BP (2, violet), BkF (3, violet), IP (4, yellow), BbF (5, turquoise) and BaP (6, violet).

spots. In a dark cabinet plates with standard chromatograms can be viewed simultaneously with a sample chromatogram to estimate the concentration visually. A typical standard chromatogram is given in **Figure 1**. A chromatogram of a river water sample is shown in **Figure 2**.

Quantification of the Spots using a Scanning Fluorimeter

It is also possible to evaluate the chromatograms using a scanning fluorimeter, but this procedure is very time-consuming and therefore in most cases only semiquantitative evaluation by visual estimation is used.

Table 2 Suitable excitation and emission wavelengths

Compound	*Excitation wavelength (nm)*	*Emission wavelength (nm)*
Fluoranthene	365	462
Benzo(b)fluoranthene	302	452
Benzo(k)fluoranthene	302	431
Benzo(a)pyrene	297	405 or 430
Benzo(ghi)perylene	302	419 or 407
Indeno(1,2,3-cd)pyrene	300	500

For the scanning method, first the coordinates defining each spot are recorded using a standard plate. The instrument settings (slit height and width, wavelengths, etc.) required to achieve good curves must be determined individually for the available equipment, because these settings will vary with the type of instrument. For guidance, suitable excitation and emission wavelengths are given in **Table 2**.

The standard and the sample plates are scanned using the same instrument settings. For each spot on the plates the area of the peak is measured. The mass of each individual PAH in the sample can then be calculated by comparison with the standard.

Determination of PAH by HPTLC with Fluorescence Detection

The two methods described below are applicable to the determination of the six selected PAH in drinking

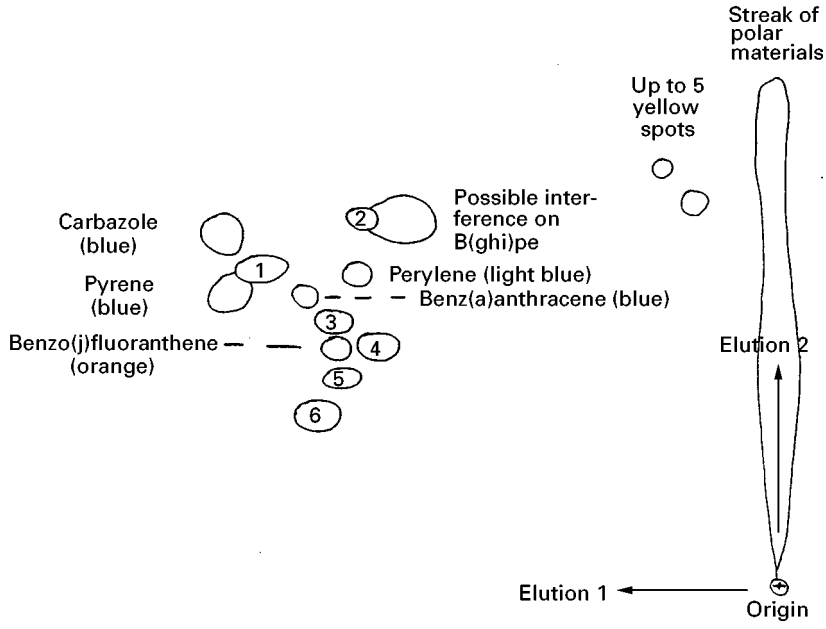


Figure 2 River water chromatogram viewed under UV light with F (1, turquoise). BP (2, violet), BkF (3, violet), IP (4, yellow), BbF (5, turquoise) and BaP (6, violet).

water, ground waters and moderately polluted surface waters. For other samples, containing a lot of interfering substances, specially developed clean-up procedures are necessary.

The first method uses HPTLC RP-18 plates and is a screening method for semiquantitative determination. It can be used to screen for samples with PAH concentrations near or above the limiting value, which then have to be analysed quantitatively.

For the quantitative method, using caffeine-impregnated HPTLC silica gel plates, the chromatograms are evaluated by *in situ* fluorescence measurement at constant or differing wavelength combinations. This method has a working range of 40–240 ng L^{-1} for the sum of the six PAH.

PAH are extracted from the water sample by liquid–liquid extraction as described above. The extract is evaporated to dryness and the residue taken up in a small defined volume of solvent. An aliquot of this extract is applied on to the HPTLC plate. A more detailed description of the methods is given below.

HPTLC Method with RP-18 Plates

Plate Material

HPTLC-pre-coated RP-18 plates, preferably with fluorescence indicator, are used. Plates with concentrating zones may also be used. RP-18 materials with different degree of coverage (carbon loading) are commercially available and, depending on this, different mobile phases have to be used.

Application of Sample Extracts and Standard Solutions

Several samples may be analysed simultaneously on one HPTLC plate, together with two or more reference solutions of different concentrations. For screening purposes it is also possible to apply sample extracts and reference solutions on both ends of the HPTLC plate, provided the plate is developed in a horizontal development chamber.

Aliquots, e.g. 25%, of the total sample extracts (40–120 μL) are applied either by means of an automated volume-dosing device or by hand as bands or spots. For the application of spots HPTLC RP-18 plates with a concentrating zone are preferred and the volumes should not be more than 10 μL. If using band application, the bands should have a length of 7 mm and the intervals should be 3 mm. For band application the extract should not be too concentrated, and it is advantageous to apply higher volumes, e.g. 30 μL.

Development of the Plates

The development is performed in a development chamber for low consumption of mobile phase, suitable for trace analysis.

Depending on the carbon loading, one of the following mobile phases is used:

- a mixture of acetonitrile, 2-propanol and methanol (1 + 2 + 1) for high carbon loading C_{18} material
- a mixture of acetonitrile, water and methanol (10 + 2 + 10) for low carbon loading C_{18} material

The chromatogram is developed at room temperature either vertically in a trough chamber or horizontally in a horizontal development chamber, without chamber saturation. Using a trough chamber, the run time for a migration distance of 6.5 cm is about 20 min. In a horizontal development chamber, the run time for a migration distance of 6 cm is about 20 min, and for 4.5 cm about 15 min.

After development the plate is dried for 2 min in a stream of air at ambient temperature.

Visual Evaluation of the Chromatograms

If the dried plate is viewed under an ultraviolet lamp at 366 nm only three to four fluorescing zones can be recognized. **Figure 3** shows two examples: first, a chromatogram of the six PAH on a plate with high carbon load C_{18} material, with a concentrating zone,

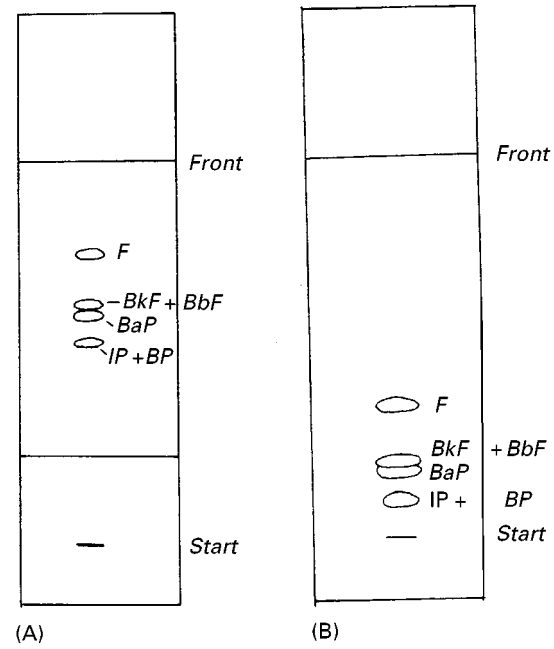


Figure 3 Chromatogram of the six PAH on HPTLC RP-18 plates. (A) high carbon load C_{18} material, plate with concentrating zone; (B) low carbon load C_{18} material.

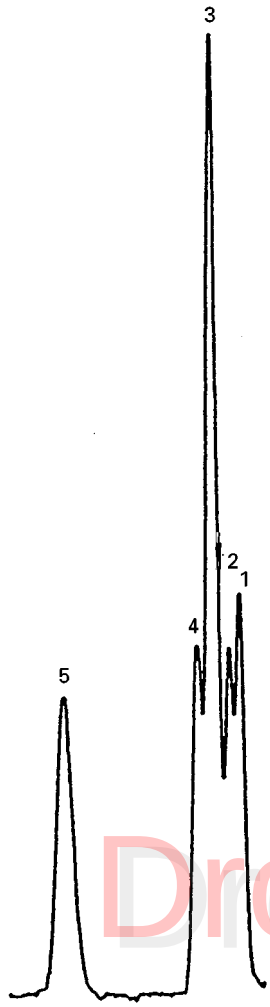


Figure 4 Fluorescence scan of a chromatogram track with 2 ng per spot of BP, IP, BaP, BbF, BkF and 10 ng per spot of F. The separation from the start to the front is IP (1), BP (2), BaP and BkF (3), BbF (4) and F (5).

and second, a chromatogram of the six PAH on a plate with low carbon load C_{18} material.

This separation is sufficient to evaluate the sample chromatograms visually by comparison with standard chromatograms. The individual PAH can be identified by colour, R_F value, and position relative to the reference chromatogram. The colours of the PAH are: F = light blue, BkF = dark blue, BbF = blue, BaP = violet, IP = light yellow and BP = violet. The concentration of the PAH in the sample extracts can be estimated by the fluorescence intensity of the zones. If, as in most drinking and ground waters, the fluorescence intensity is not stronger than the fluorescence of a corresponding standard, the PAH concentration lies below the limiting value, and therefore quantitative measurement is not necessary.

Influence of Temperature

Working at lower temperatures leads to better separation. The six PAH can be separated at $-20^{\circ}C$ into five fractions. **Figure 4** shows the fluorescence scan after chromatographic development on a high carbon load C_{18} material. The separation is still not satisfactory as BaP and BkF are not separated. Due to this, the described separation technique is not suitable for quantitative analysis of the individual compounds.

HPTLC Method with Caffeine-impregnated Silica Gel Plates

The ability of PAH to form charge transfer complexes can be successfully employed when applied to silica gel thin layers impregnated with different electron acceptors, such as caffeine. In the following paragraphs charge transfer chromatography on caffeine-impregnated HPTLC silica gel plates is described. The chromatographic separation at room temperature is an alternative method to the screening method on HPTLC RP-18 plates. If the development is performed at $-20^{\circ}C$, the six PAH can be easily separated into six fractions and the chromatograms can be evaluated quantitatively.

Plate Material

Caffeine-impregnated HPTLC silica gel 60 plates are commercially available, but the impregnation can also be carried out in the laboratory.

For caffeine impregnation HPTLC silica gel 60 plates are dipped for 4 s into a solution containing 4 g caffeine in 96 g dichloromethane and then dried at $110^{\circ}C$ for 30 min. Prior to use, the plates should be cleaned by running a blank chromatogram to the upper edge, using dichloromethane. This pre-washing step is especially recommended if quantitative determination is intended. Afterwards the plates need to be reactivated by heating at $110^{\circ}C$ for 30 min. The activated plates should be stored in a desiccator until use.

Application of the Sample Extracts and Standard Solutions

Aliquots of sample extracts and standard solutions are applied on to the same plate, preferably as bands, using an automated volume-dosing device.

For screening purposes, extracts and standard solutions are applied as bands or spots on both ends of the HPTLC plate, provided the plate is developed in a horizontal development chamber.

For quantitative analysis only band application is recommended. The bands should have a length of 7 mm and the intervals should be 3 mm. The applied volumes should be between 10 and 30 μL.

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Development of the Plates at Room Temperature

After application of the samples, the plate is preconditioned for 30 min over water. Then the chromatogram is developed vertically or horizontally in a development chamber without chamber saturation, using a mixture of diisopropyl ether and *n*-hexane (4 + 1) as mobile phase. Using a trough chamber, the run time for a migration distance of 6.5 cm is about 25 min; in a horizontal development chamber, the run time for a migration distance of 6.5 cm is about 15 min, and for 4.5 cm about 10 min. After development the plate is dried for 2 min in a stream of air at ambient temperature. Then, in order to stabilize the fluorescence intensity of the chromatogram zones for more than 1 h, the plate is dipped into a solution of liquid paraffin–*n*-hexane (1 + 2) for 2 s, then dried again for 2 min. This not only leads to stabilization but in addition the fluorescence intensity is doubled for F, BkF, BbF and IP, and enhanced by a factor of 5 for BaP and BP.

Visual Evaluation of the Chromatograms

If the dried plate is viewed under an ultraviolet lamp at 366 nm, five fluorescing zones can be recognized (**Figure 5**A). The individual PAH can be identified by colour, R_F value and relative position to the reference chromatogram. The concentration of the PAH in the sample extracts can be estimated by the fluorescence intensity of the zones. The fluorescence scan of the chromatogram (**Figure 5**B) shows that there is no baseline separation between BaP and BbF. Therefore a quantitative evaluation of the chromatograms, developed at room temperature, would not yield reliable results for these substances.

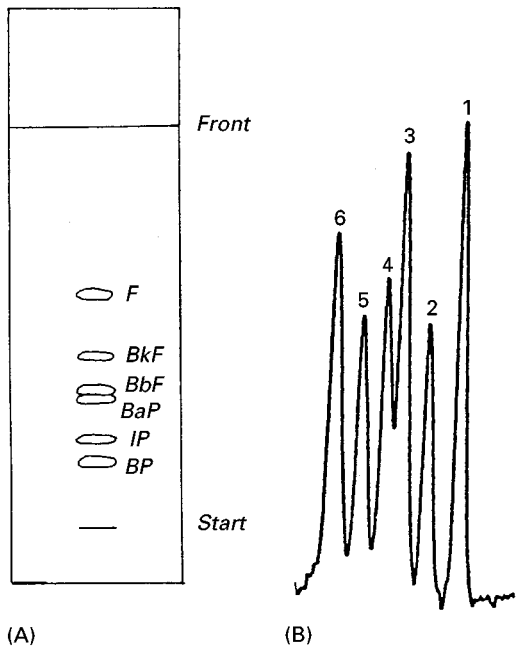

Figure 5 (A) Chromatogram of the six PAH on a caffeine-impregnated HPTLC silica gel plate. Development at room temperature. (B) Florescence scan of a chromatogram track with 2 ng per spot BP (1), IP (2), BaP (3), BbF (4) and BkF (5) and 10 ng per spot of F (6). Development at room temperature.

Development of the Plates at − 20°C

After sample application, the plate has to be precooled in a freezer cabinet at − 20°C for 20 min. Then the chromatogram is developed vertically in a trough chamber without chamber saturation at − 20°C with dichloromethane as mobile phase. The chamber has to be kept, for equilibration, at − 20°C for at least 60 min before the analysis. The run time for a migration distance of 6.5 cm is about 20 min. Development in a horizontal development chamber does not give reproducible results.

After development the plate is dried for 2 min in a stream of air at ambient temperature. Then, in order to stabilize and increase the fluorescence intensity of the chromatogram zones, the plate is dipped, as described above, into a solution of liquid paraffine/*n*-hexane.

Fluorescence Densitometric Evaluation

Using the separation procedure at − 20°C described above, the six PAH can be completely separated. Therefore this procedure is well suited for quantitative determination.

The chromatograms can be evaluated by measuring either the peak height or peak area using a scanner at an excitation wavelength of 366 nm and a fluorescence wavelength of 400 nm (edge filter). Standard chromatograms are evaluated to calculate a calibration function. The fluorescence intensity of the chromatogram zones is linearly related to the amount of PAH applied, up to 12 ng per spot for fluoranthene and up to 2.4 ng per spot for the other five PAH. The precision of the calibration functions is excellent (coefficients of variation between 1.7% and 3.8%).

If applying spots, the slit of the scanner should be broader than the largest zone of the chromatogram in the *x*-direction. If applying bands, the recommended slit width in the *x* direction is 1/2 to 2/3 of the band length. In the *y* direction, the slit should not be smaller than 0.3 mm.

Figure 6A shows the position of the six PAH on the caffeine-impregnated HPTLC silica gel plate. The fluorescence chromatogram (Figure 6B) demonstrates that the described procedure yields nearly baseline separation.

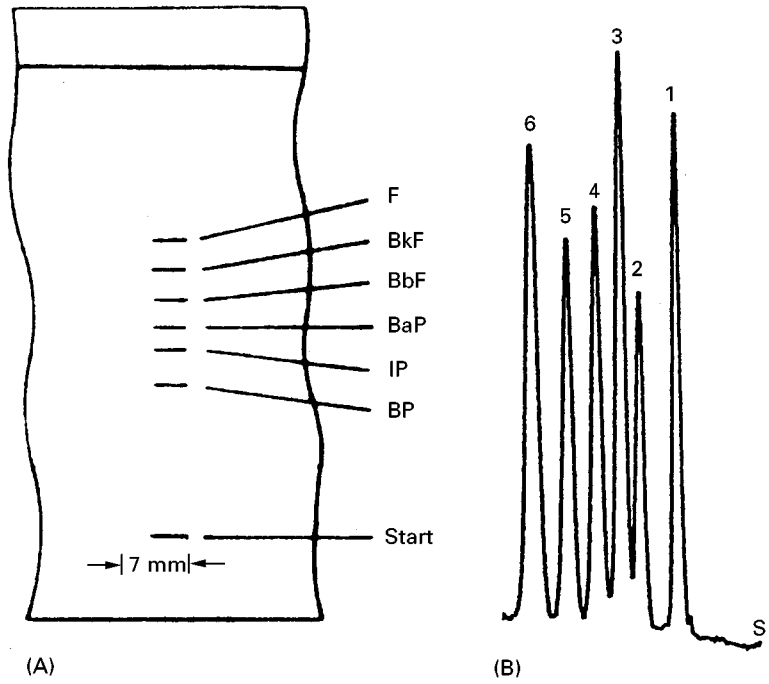

Figure 6 (A) Chromatogram of the six PAH on caffeine-impregnated silica gel plates, developed at − 20°C; (B) Fluorescence chromatogram of a track with 2 ng per spot BP (1), IP (2), BaP (3), BbF (4) and BkF (5) and 10 ng per spot of F (6); development at − 20°C.

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Figure 7 shows the chromatogram of a ground water sample, in which all six PAH were found, plus one unknown fluorescing substance. This was an ideal sample, but other fluorescing substances may interfere with the determination of the PAH. In such cases the PAH can be selectively detected, using different excitation and emission wavelengths.

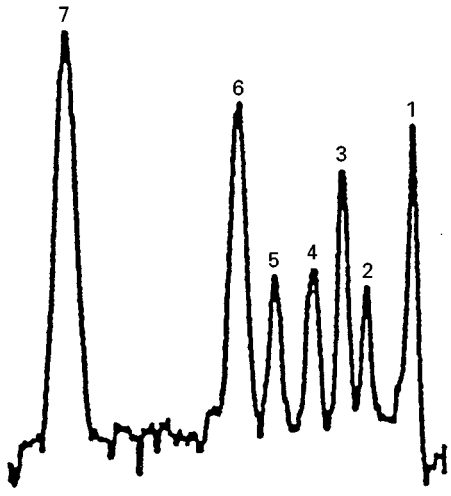

Figure 7 Fluorescence scan of a ground water sample with BP (1), IP (2), BaP (3), BbF (4), BkF (5), F (6) and an unknown substance (7).

Spectroscopic Identification

As the six PAH show different optical properties it is possible to detect them selectively. With HPTLC, which is characterized by offline detection, it is easy to repeat the scanning procedure several times using different easily selectable fluorimetric conditions within a short time period.

For example **Figure 8** shows the evaluation of the same chromatogram at different excitation wavelengths and emission filters. By spectroscopic selection it is possible to guarantee the correctness of qualitative and quantitative results.

Conclusion

TLC is an efficient and versatile analytical method. The costs per sample are low and, because many samples can be analysed in one development step, the time to achieve results for a series of samples is comparably short. Although TLC has many advantages, this method is not often used for the analysis of PAH; the equipment for quantitative HPTLC is not widespread in water laboratories and only the screening method to control the observance of the limiting value for drinking water has found greater acceptance. Many laboratories prefer high performance liquid chromatography or gas chromatography for

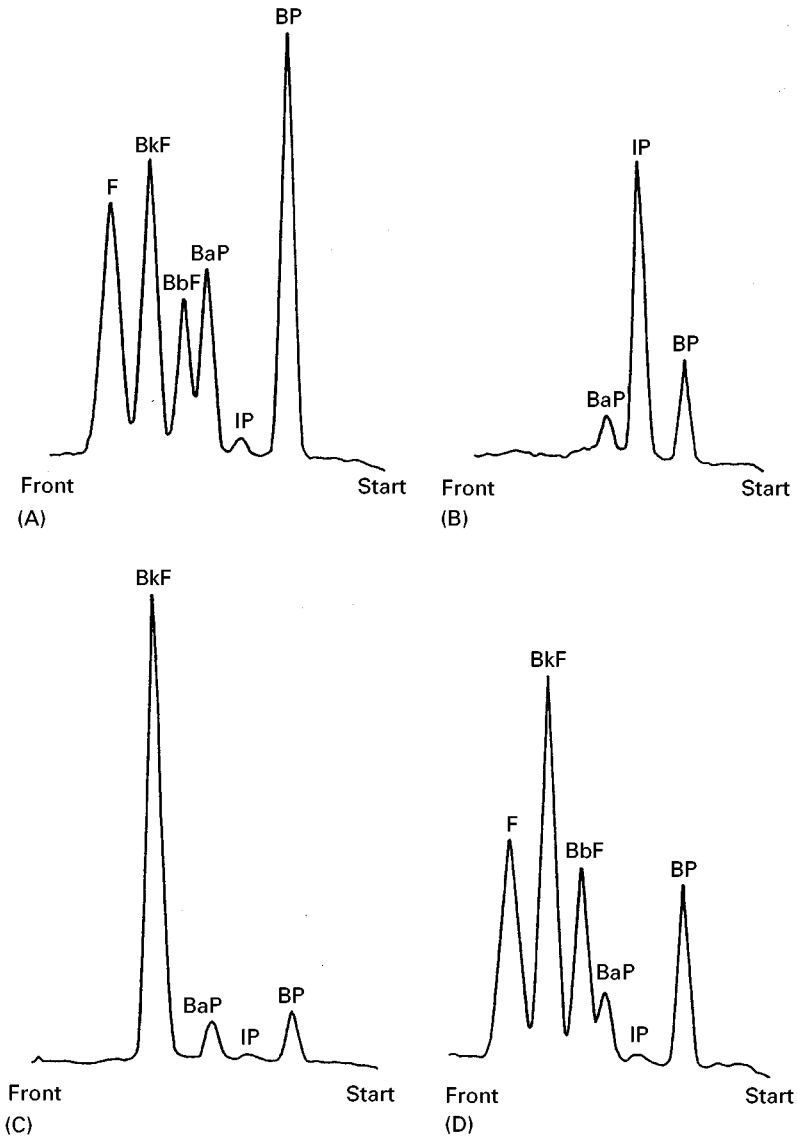

Figure 8 Fluorescence scan of the same chromatogram track. Selective detection using different excitation (λ_{exc}) and fluorescence (λ_{fl}) wavelengths: (A) $\lambda_{exc} = 365$ nm; $\lambda_{fl} = 436$ nm; (B) $\lambda_{exc} = 436$ nm; $\lambda_{fl} = 578$ nm; (C) $\lambda_{exc} = 405$ nm; $\lambda_{fl} = 436$ nm; (D) $\lambda_{exc} = 334$ nm; $\lambda_{fl} = 436$ nm.

the quantitative determination of PAH, because the separation efficiency of these methods is better and they can be automated. Therefore further development in the field of PAH determination with TLC cannot be expected, although modern HPTLC offers a lot of possibilities.

See also: **II/Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Instrumentation; Layers; Modes of Development: Conventional; Spray Reagents. **III/Impregnation Techniques: Thin-Layer (Planar) Chromatography: Polycyclic Aromatic Hydrocarbons:** Gas Chromatography; Liquid Chromatography; Supercritical Fluid Chromatography.

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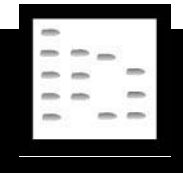
POLYETHERS: LIQUID CHROMATOGRAPHY



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Introduction

Before chromatography became an efficient tool for polymer fractionation, classic extraction procedures played a dominant role. These techniques comprise, e.g., dissolution and precipitation, depending on the solvent–non-solvent ratio and M_r, extraction of native polymer or polymer adsorbed onto a solid support with organic solvents of increasing dissolution capability, and partition between two immiscible liquids. In contrast, Baker–Williams and temperature rising elution fractionation (TREF), also used in polymer chemistry, are chromatographic techniques. Although separation of polymers by means of various classic extraction procedures are still in use, chromatographic characterization now plays the dominant role and affords an optimum degree of structural information. In this respect, polyethers of the polyethylene glycol (PEG), polypropylene glycol (PPG) and polybutylene glycol (PBG) family, all extensively used in different fields of chemistry and engineering, have been selected as model compounds for separation of polymers because they differ widely in chemical properties and polarity, ranging from hydrophilic (PEGs) to hydrophobic (PBGs) in either native form or mono-(di-)O-alkyl(arylalkyl) (**Figure 1**A–C) and amino-terminal derivatives (**Figure** 1B). For this reason, they comprise a group of polymers accessible to a broad range of chromatographic separation techniques including high performance liquid chromatography (HPLC), size-exclusion chromatography (SEC), thin-layer chromatography (TLC), supercritical fluid chromatography (SFC) and capillary zone electrophoresis (CZE). Gas chromatography (GC) only provides separation of the low-molecular-weight (M_r) members of polyethers with upper limits of M_r of approximately 600. TLC and SFC are of minor importance and are not considered. Although extensively used for determination of M_r values, SEC is also excluded because it exhibits only moderate resolution and does not permit differentiation of the individual types of polyether on the basis of the underlying chemistry.

This survey gives a short overview of the current state of HPLC technology of polyethers and deriva-

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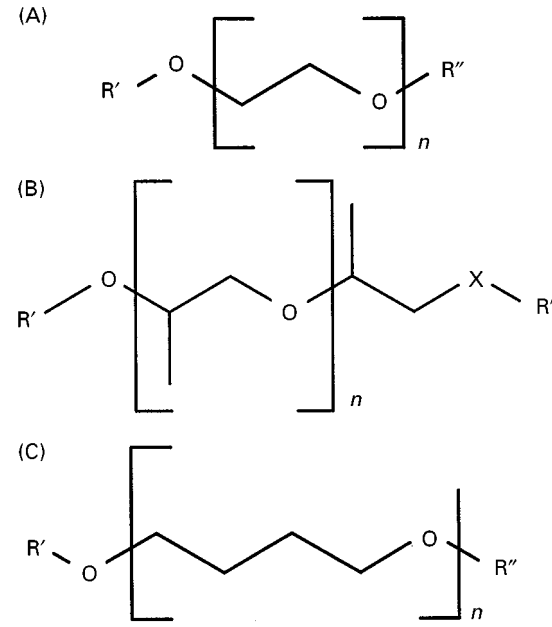

Figure 1 Structures of polymers. (A) R′, R″ = H, n-alkyl, alkyl–C=O, aryl(alkyl); R′ = R″, R′ ≠ R″; (B) R′ = H, glyceryl, trimethylolpropyl; R″ = H (X = O), H_2 (X = N); (C) R′ = R″ = H, alkyl, aryl(aroyl); R′ = R″, R′ ≠ R″.